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## Review

## A new twist to coiled coil

Elisabeth Le Rumeur<sup>a,b,c</sup>, Jean-François Hubert<sup>a,b,c</sup>, Steve J. Winder<sup>d,\*</sup>

<sup>a</sup> Université Européenne de Bretagne (UEB), 35000 Rennes, France

<sup>b</sup> UMR-CNRS 6290, Equipe Structures et Interactions Moléculaires, CS 34317, 35043 Rennes, France

<sup>c</sup> Université de Rennes 1, 35043 Rennes, France

<sup>d</sup> Department of Biomedical Science, University of Sheffield, Sheffield S10 2TN, UK

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## ABSTRACT

**Spectrin repeats have been largely considered as passive linkers or spacers with little functional role other than to convey flexibility to a protein. Whilst this is undoubtedly part of their function, it is by no means all. Whilst the overt structure of all spectrin repeats is a simple triple-helical coiled coil, the linkages between repeats and the surface properties of repeats vary widely. Spectrin repeats in different proteins can act as dimerisation interfaces, platforms for the recruitment of signalling molecules, and as a site for the interaction with cytoskeletal elements and even direct association with membrane lipids. In the case of dystrophin several of these functions overlap in the space of a few repeats.**

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### 1. Introduction

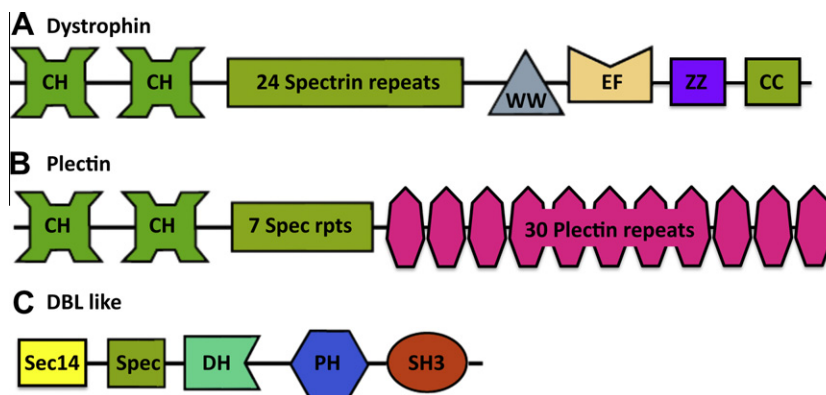
The presence of multiple coiled coil modules, and in particular spectrin repeats is a common feature of cytoskeletal linking proteins, or cytolinkers. Cytolinker was a term originally coined to describe plectin [1] but has now been adopted to describe a broader group of proteins including plakins, nesprins and spectrin family proteins, all of which contain multiple spectrin repeats. The presence of spectrin repeats in these cytolinkers can mediate self association and permits flexible and perhaps extensible linkages so the proteins can connect between different cytoskeletal filament systems, or between cytoskeletal systems and cellular membranes. However in recent years evidence has emerged of a role for the spectrin repeat as a binding interface in its own right, making direct protein–protein interactions and in some cases protein–lipid interactions.

### 2. Overview of the family of spectrin repeat containing proteins

More than 97% of known spectrin repeat containing proteins are found in metazoans with over two thirds of those in chordates [2]. Despite scattered examples of spectrin repeats in all other kingdoms, this would tend to suggest that the spectrin repeat arose with the evolution of the animal kingdom. With the exception of one or two outliers, as mentioned above, most proteins are considered as cytolinkers and can be broadly grouped into 2 or 3 families depending on ones perspective. The eponymous family from which the repeat derives its name includes the proteins  $\alpha$ -actinin, spectrins themselves and dystrophin and utrophin. These proteins share a variable number of spectrin-like repeats, from 4 in  $\alpha$ -actinin to 24 in dystrophin, and depending on the protein also have an amino-terminal actin binding domain comprising tandem CH domains and carboxy-terminal calcium binding EF hands. In addition different family members have acquired additional domains specific to their cellular functions, including PH, SH3, WW and ZnF (Fig. 1A). Full listings and domain compositions can be found in several online databases for example SMART, PFAM and Domain Club (<http://smart.embl-heidelberg.de/>, <http://pfam.org>).

\* Corresponding author.

E-mail address: [s.winder@sheffield.ac.uk](mailto:s.winder@sheffield.ac.uk) (S.J. Winder).



**Fig. 1.** Schematic representation of selected members of spectrin repeat containing proteins from the three main families. (A) Dystrophin; (B) Plectin and (C) DBL-like. Modules are coded according to the style and nomenclature of Pawson and Nash [53] with the addition of the Sec14 domain [54] (yellow box) and ZZ domain [55] (purple box) and plectin repeats (magenta heptagons). Definitive publications to the modules shown above are: CC [56] CH [57] DH [58] EF [59] PH [60,61] Plec [62] SH3 [63,64] Spec [7] WW [65].

sanger.ac.uk/, <http://pawsonlab.mshri.on.ca/DomainClub/domain-Club.php>). For the purposes of this review we will discuss in more detail new functions and properties of the spectrin repeats of the spectrin family member dystrophin.

The plakin family of proteins is characterised by the presence of plectin repeats and spectrin repeats often interspersed with other sequences with a propensity to form dimeric coiled coils. Other modules found in plakins include tandem CH domains, EF hands, SH3 and KASH again dependent on cellular function (Fig. 1B). Plakin family proteins function to interconnect different cytoskeletal filament networks with each other and directly to membranes or membrane associated structures. Depending on their domain composition they variously connect intermediate filaments via their plectin repeats (e.g. desmoplakin, plectin and some nesprin isoforms), actin filaments via their CH domains (plectin, MACF1 and some nesprin isoforms) and microtubules (plectin, MACF1). In turn they associate with other membrane anchored proteins in the plasma membrane at adhesion sites such as costameres, desmosomes and hemidesmosomes. The plakins also have roles in organelle positioning including mitochondria and Golgi, and in the maintenance of nuclear membrane connectivity to the cytoskeleton as well as the structure of the nuclear lamina. Readers are referred to more authoritative reviews for details of these functions [3,4].

In addition to multiple repeated copies of spectrin repeats in the cytolinker proteins mentioned above, spectrin repeats also occur sparsely in some Rho family guanine nucleotide exchange factors (RhoGEFs) including the RhoGEF DBL, its big sister MCF2L and more distant relatives such as trio and kalirin. With the exception of DBL itself, these all contain an amino terminal SEC14 domain, followed by one or more spectrin repeats followed by one or more copies of the DH and PH domains characteristic of GEFs also with SH3 and S/T kinase domains (Fig. 1C). Loss of the spectrin repeat in kalirin alters its effects on actin based structures such as dendritic spines [5]. In a similar manner deletion of the spectrin repeat from Dbl contributes to its oncogenic potential by removing binding sites for HSc70 and a ubiquitin ligase that serve to maintain low steady state levels of the protein [6]. Thus the functions of the spectrin repeats in kalirin and Dbl appear to control GEF function and/or targeting of the GEF activity.

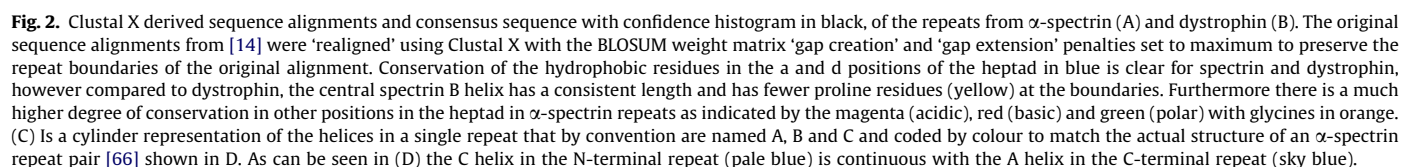
### 3. Spectrin family repeat structure

The core elements of the spectrin repeat are a triple-helical coiled-coil bundle, with the 3 helices forming the domain gently

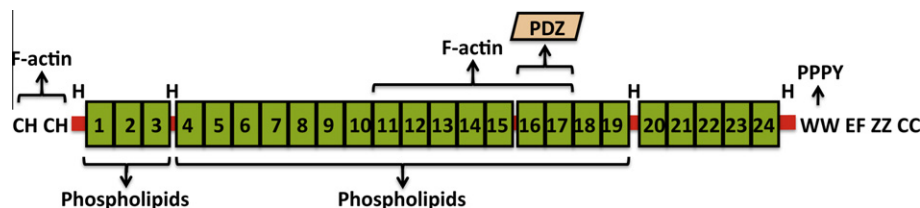
curving and wrapping around each other in a left-handed supercoil (Fig. 2). The archetypal spectrin repeat structure obtained from the direct protein sequencing of spectrin in the early nineteen eighties revealed a repeating 106 amino acid sequence with conserved periodic hydrophobic and charged residues [7]. Predicted sequences for dystrophin and  $\alpha$ -actinin obtained slightly later by DNA sequencing also revealed similarities to the repeating regions of spectrin [8–10] but with slightly different average repeat lengths of 122 and 109 residues for  $\alpha$ -actinin and dystrophin, respectively. An evolutionary relationship has also been proposed for this protein family from a likely  $\alpha$ -actinin ancestor and subsequent diversification to spectrins and then dystrophin/utrophin [11–13]. The repeats of  $\alpha$ -actinin and spectrin are known to form dimers, indeed, the regular repeat length and conserved surface charge particularly in the e and g positions in the heptad lends itself to dimerisation. By convention the amino acids in helices are lettered from a to g to represent the 7 residues per two turns of the helix, i.e. the heptad. Whilst hydrophobic residues at the a and d positions in the heptad, a hallmark of a triple-helical coiled coil, are conserved in dystrophin and its autosomal homologue utrophin, they both lack the conservation of repeat length and charged residues at the e and g positions in the heptad to form stable dimers [14–16] (Fig. 2).

The first structures of single spectrin repeats as predicted [17] revealed tight triple  $\alpha$ -helical coiled-coils [18,19]. However these single repeat structures did not reveal the true spectrin coiled coil structure due to either the long helix folding back on itself or the repeat dimerising. It was only later when multiple repeats were solved that the continuous relationship between the helices in the repeat junction was elucidated [20–22] (Fig. 2). Furthermore the crystal structures of a repeat pair from  $\alpha$ -spectrin in multiple crystal forms revealed the potential flexibility of spectrin repeats [21] whereas structures of the four spectrin repeats from  $\alpha$ -actinin yielded a rather rigid dimerised structure [20,22]. As noted above, dystrophin repeats are more variable in length and have more frequent insertions in the helices [14] (Fig. 2). In addition, and in contrast to spectrin molecules, four predicted hinges separating the rod region into three sub-regions were speculated to confer additional flexibility to the molecule (Fig. 3) [9]. The alpha-helical nature of the dystrophin spectrin-like repeats was confirmed, however additional residues were required to extend the helices into the adjoining helices in order to produce a stable fold [23–25]. These studies suggested that the dystrophin repeats may fold in an overlapping or nested manner with the structural integrity of each repeat being reliant in part on its neighbours [16,26,27]. This





inter-helical linkers were for the majority helical, but for some containing proline residues, these regions were modelled as small loops and interpreted as likely points of additional flexibility [30]. The same points are also observed between the equivalent repeats in utrophin but not in any spectrins [30]. Furthermore, analysis of the surface properties of the repeat pairs revealed considerable differences in hydrophobic and electrostatics surfaces [30], a degree of variability which is in contrast to the uniformity of



**Fig. 3.** Schematic overview of spectrin repeat interactions in dystrophin. In keeping with established nomenclature [67] 4 hinge regions (H) are shown in red, however a fifth extended region between repeats 15 and 16 which should probably be considered a hinge [68] is also depicted in red. Precise molecular details of which single repeats interact with F-actin, phospholipids and the PDZ domain of nNOS are not known, and are therefore bracketed by repeat number. As can be seen repeats 16 and 17 have overlapping functions as discussed in the text. The key interactions of the tandem CH domains with F-actin [69] and the WW domain with the PPPY of  $\beta$ -dystroglycan [70] are also represented.

structure and the probable uniformity of surface properties of spectrin repeats (Fig. 2A). In turn it is this variability that is likely to confer unique properties to the dystrophin repeats that will be discussed in more detail below.

#### 4. Spectrin-repeat interactions in dystrophin

##### 4.1. Actin binding

Many members of the spectrin-repeat containing protein family interact with the actin cytoskeleton. In most cases direct interaction with F-actin is mediated by a pair of amino-terminal CH domains, a well characterised actin binding module found in a large number of F-actin binding proteins [31]. However in addition to the CH domain mediated actin binding activity, the Ervasti lab also characterised a second major actin binding region in dystrophin situated in repeats 11–17 [32,33] (Fig. 3). Whilst the affinities of either the CH domain actin binding site and the dystrophin-repeat actin binding site are individually relatively low, when present together in the same molecule they act synergistically to provide a high affinity interaction with F-actin [33,34]. Despite the high degree of sequence homology over the whole length of dystrophin and utrophin, utrophin does not share the same actin binding functions in the central repeats [35]. Repeats 11–17 of dystrophin are relatively basic in nature which facilitates an electrostatic interaction with F-actin, whereas the equivalent repeats in utrophin are acidic. However, the addition of up to ten canonical repeats to the utrophin CH domains stabilised the interaction between utrophin and actin increasing the affinity by up to 20-fold [36]. The addition of up to two repeats to the  $\beta$ -spectrin actin binding domain had a similar effect [37], but in both the case of  $\beta$ -spectrin and utrophin the repeats have no intrinsic actin binding properties alone and absolutely require the CH domains. Despite both dystrophin and utrophin being able to interact along the length of actin filaments through at least half of their molecular length, the binding was not competitive, with both proteins able to interact with F-actin simultaneously [34]. One consequence of the binding of dystrophin or utrophin laterally on actin filaments is to greatly increase the torsional flexibility of actin [38], a property that could be of great functional significance for the protection of the sarcolemma during the deformation induced by contraction and relaxation.

##### 4.2. Lipid Binding

Mapping of the lipid binding properties of dystrophin repeats revealed that repeats 1–19 can bind to anionic lipids whereas repeats 20–24 do not [39,40]. This has led to the suggestion that dystrophin may in fact associate directly with the membrane and for part of its repeat region lies along the membrane, reviewed in [29]. Furthermore biophysical studies of the lipid binding repeats

1–3 and the non-lipid-binding repeats 20–24 revealed that the interaction of repeats 1–3 with membrane lipids was largely electrostatic with no modification of the helical secondary structure of the protein [41]. This is in contrast to the interaction of repeat 14 of  $\beta$ -spectrin with lipids, which undergoes significant unfolding in order to interact with phospholipids [42]. Of the dystrophin repeats that do interact with lipids, repeats 11–15 are unique in that they are able to bind to both anionic and zwitterionic lipids [40]. Interestingly repeats 11–17 have also been demonstrated to interact with F-actin [33] (see above). A more detailed biophysical analysis of repeats 11–15 revealed that this region is able to create very strong protein networks at the interface of both anionic and zwitterionic lipid membranes [43]. This confirmed the amphiphilic nature of these repeats and their propensity to spread onto the membrane surface dependent on the surface pressure and the lipid packing. Given that the interaction of dystrophin is dependent on the surface pressure of the lipid monolayer, these properties are likely to have important biological consequences as they could relate to the association of dystrophin with the sarcolemma during changes in surface pressure due to the muscle contraction-relaxation cycle.

The ability of repeats 11–15 to bind to actin and to lipid membranes suggests a possible role for these associations in stabilising the membrane. Neither dystrophin repeats 11–15 nor F-actin alone has any significant effect on the viscoelasticity of the lipid membrane, however when actin and dystrophin repeats 11–15 are added together there is a highly significant increase in membrane stiffness that is dependent on dystrophin repeat concentration and actin polymerisation, as well as lipid type and surface pressure [44]. Taken together these findings present a new paradigm for the functional role of dystrophin in protecting the membrane from contraction-associated damage by forming a more continuous bridge between sarcolemma and underlying cytoskeletal elements such as actin, a role mediated by the dystrophin repeats.

##### 4.3. nNOS

Neuronal nitric oxide synthase (nNOS) is a sarcolemma associated enzyme important for skeletal muscle vasomodulation [45]. nNOS is lost from the sarcolemma due to the loss of dystrophin and associated proteins in Duchenne muscular dystrophy [46]. Evidence from Becker muscular dystrophy patients who lack only part of the dystrophin gene, and from the *mdx* mouse, a model of Duchenne muscular dystrophy, highlighted a requirement for the repeat region of dystrophin [47–49]. However experiments in *mdx* mice also highlight a requirement for syntrophin, one of the dystrophin associated proteins [48], though syntrophin alone is not sufficient to restore nNOS to the sarcolemma [49]. More detailed analysis of a potential ternary complex between nNOS, dystrophin repeats and syntrophin reveals a direct interaction between the PDZ domain of nNOS and repeats 16 and 17 of dystrophin [50].



## 5. Biomedical significance

A significant proportion of DMD mutations arise in the repeat region of dystrophin, many as a consequence of deletions of one or several exons and nonsense mutations. Recent advances in molecular medicine now make it theoretically possible to correct these mutation using exon-skipping strategies, see [51] for a recent comprehensive review. However for these strategies to be successful, one not only requires a knowledge of genomic structure and protein structure but also protein function. The importance of maintaining the phasing of the repeats in dystrophin has been realised for some time [14], however it is only more recently that the importance of which dystrophin repeats are required for a functional rescue of dystrophic muscle has become apparent, reviewed in [29]. As an aide to basic and clinical scientists alike we have developed an eDystrophin database devoted to the analysis and human DMD mutations and prediction of resulting protein structure with particular emphasis on the repeat region. The eDystrophin database is available: <http://edystrophin.genouest.org/> [52]. As is apparent from the analysis described above, the overlapping functions of actin binding, phospholipid binding and nNOS binding makes the restoration of dystrophin repeats 11–17 critical to the success of any therapy.

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## References

- [1] Wiche, G. (1998) Role of plectin in cytoskeleton organization and dynamics. *J. Cell Sci.* 11, 2477–2486.
- [2] Letunic, I., Doerks, T. and Bork, P. (2011) SMART 7: recent updates to the protein domain annotation resource. *Nucleic Acids Res.* 40, D302–D305.
- [3] Mellad, J.A., Warren, D.T. and Shanahan, C.M. (2011) Nesprins LINC the nucleus and cytoskeleton. *Curr. Opin. Cell Biol.* 23, 47–54.
- [4] Boyer, J.G., Bernstein, M.A. and Boudreau-Larivière, C. (2010) Plakins in striated muscle. *Muscle Nerve* 41, 299–308.
- [5] Schiller, M.R. et al. (2008) Autonomous functions for the Sec14p/spectrin-repeat region of Kalirin. *Exp. Cell Res.* 314, 2674–2691.
- [6] Kamynina, E., Kauppinen, K., Duan, F., Muakkassa, N. and Manor, D. (2007) Regulation of proto-oncogenic dbp by chaperone-controlled, ubiquitin-mediated degradation. *Mol. Cell Biol.* 27, 1809–1822.
- [7] Speicher, D.W. and Marchesi, V.T. (1984) Erythrocyte spectrin is comprised of many homologous triple helical segments. *Nature* 311, 177–180.
- [8] Baron, M.D., Davison, M.D., Jones, P. and Critchley, D.R. (1987) The sequence of chick  $\alpha$ -actinin reveals homologies to spectrin and calmodulin. *J. Biol. Chem.* 262, 17623–17629.
- [9] Koenig, M., Monaco, A.P. and Kunkel, L.M. (1988) The complete sequence of dystrophin predicts a rod-shaped cytoskeletal protein. *Cell* 53, 219–226.
- [10] Davison, M.D. and Critchley, D.R. (1988) Alpha-actinins and the DMD protein contain spectrin-like repeats. *Cell* 52, 159–160.
- [11] Dubreuil, R. (1992) Structure and evolution of the actin crosslinking proteins. *BioEssays* 13, 219–226.
- [12] Pascual, J., Castresana, J. and Saraste, M. (1997) Evolution of the spectrin repeat. *BioEssays* 19, 811–817.
- [13] Thomas, G., Newbern, E., Korte, C., Bales, M., Muse, S., Clark, A. and Kiehart, D. (1997) Intragenic duplication and divergence in the spectrin superfamily of proteins. *Mol. Biol. Evol.* 14, 1285–1295.
- [14] Winder, S.J., Gibson, T.J. and Kendrick-Jones, J. (1995) Dystrophin and utrophin: the missing links! *FEBS Lett.* 369, 27–33.
- [15] Winder, S.J., Gibson, T.J. and Kendrick-Jones, J. (1996) Low probability of dystrophin and utrophin coiled coil regions forming dimers. *Biochem. Soc. Trans.* 24, 280S.
- [16] Winder, S.J., Knight, A.E. and Kendrick-Jones, J. (1997) Protein Structure in: Dystrophin: gene, protein and cell biology (Brown, S.C. and Lucy, J.A., Eds.), pp. 27–55, Cambridge University Press, Cambridge.
- [17] Parry, D.A.D., Dixon, T.W. and Cohen, C. (1992) Analysis of the three- $\alpha$ -helix motif in the spectrin superfamily of proteins. *Biophys. J.* 61, 858–867.
- [18] Pascual, J., Pfuhl, M., Rivas, G., Pastore, A. and Saraste, M. (1996) The spectrin repeat folds into a three-helix bundle in solution. *FEBS Lett.* 383, 201–207.
- [19] Yan, Y., Winograd, E., Viel, A., Cronin, T., Harrison, S.C. and Branton, D. (1993) Crystal structure of the repetitive segments of spectrin. *Science* 262, 2027–2030.
- [20] Djinnovic-Carugo, K., Young, P., Gautel, M. and Saraste, M. (1999) Structure of the alpha-actinin rod: molecular basis for cross-linking of actin filaments. *Cell* 98, 537–546.
- [21] Grum, V., Li, D., MacDonald, R. and Mondragon, A. (1999) Structures of two repeats of spectrin suggest models of flexibility. *Cell* 98, 523–535.
- [22] Ylanne, J., Scheffzek, K., Young, P. and Saraste, M. (2001) Crystal Structure of the  $\alpha$ -actinin rod reveals an extensive torsional twist. *Struct. Fold Des.* 9, 597–604.
- [23] Kahana, E., Flood, G. and Gratzner, W.B. (1997) Physical properties of dystrophin rod domain. *Cell Motil. Cytoskel.* 36, 246–252.
- [24] Kahana, E. and Gratzner, W.B. (1995) Minimum folding unit of dystrophin rod domain. *Biochemistry* 34, 8110–8114.
- [25] Kahana, E., Marsh, P.J., Henry, A.J., Way, M. and Gratzner, W.B. (1994) Conformation of dystrophin structural repeats. *J. Mol. Biol.* 235, 1271–1277.
- [26] Calvert, R., Kahana, E. and Gratzner, W.B. (1996) Stability of the dystrophin rod domain fold: evidence for nested repeating units. *Biophys. J.* 71, 1605–1610.
- [27] Saadat, L., Pittman, L. and Menhart, N. (2006) Structural cooperativity in spectrin type repeats motifs of dystrophin. *Biochim. Biophys. Acta* 1764, 943–954.
- [28] Mirza, A., Sagathevan, M., Sahni, N., Choi, L. and Menhart, N. (2010) A biophysical map of the dystrophin rod. *Biochim. Biophys. Acta* 1804, 1796–1809.
- [29] Le Rumeur, E., Winder, S.J. and Hubert, J.-F. (2010) Dystrophin: more than just the sum of its parts. *Biochim. Biophys. Acta* 1804, 1713–1722.
- [30] Legrand, B., Giudice, E., Nicolas, A., Delalande, O. and Le Rumeur, E. (2011) Computational study of the human dystrophin repeats: interaction properties and molecular dynamics. *PLoS ONE* 6, e23819.
- [31] Gimona, M., Djinnovic-Carugo, K., Kranewitter, W. and Winder, S. (2002) Functional plasticity of CH domains. *FEBS Lett.* 513, 98–106.
- [32] Amann, K.J., Renley, B.A. and Ervasti, J.M. (1998) A cluster of basic repeats in the dystrophin rod domain binds F-actin through an electrostatic interaction. *J. Biol. Chem.* 273, 28419–28423.
- [33] Rybakova, I.N., Amann, K.J. and Ervasti, J.M. (1996) A new model for the interaction of dystrophin with F-actin. *J. Cell Biol.* 135, 661–672.
- [34] Rybakova, I.N., Humston, J.L., Sonnemann, K.J. and Ervasti, J.M. (2006) Dystrophin and utrophin bind actin through distinct modes of contact. *J. Biol. Chem.* 281, 9996–10001.
- [35] Amann, K.J., Guo, A.W.-X. and Ervasti, J.M. (1999) Utrophin lacks the rod domain actin binding activity of dystrophin. *J. Biol. Chem.* 274, 35375–35380.
- [36] Rybakova, I.N. and Ervasti, J.M. (2005) Identification of spectrin-like repeats required for high affinity utrophin-actin interaction. *J. Biol. Chem.* 280, 23018–23023.
- [37] Li, X. and Bennett, V. (1996) Identification of the spectrin subunit and domains required for formation of spectrin/actin complexes. *J. Biol. Chem.* 271, 15695–15702.
- [38] Prochniewicz, E., Henderson, D., Ervasti, J.M. and Thomas, D.D. (2009) Dystrophin and utrophin have distinct effects on the structural dynamics of actin. *Proc. Nat. Acad. Sci. U S A* 106, 7822–7827.
- [39] Legardinier, S. et al. (2008) Sub-domains of the dystrophin rod domain display contrasting lipid-binding and stability properties. *Biochim. Biophys. Acta* 1784, 672–682.
- [40] Legardinier, S., Raguene-Nicol, C., Tascon, C., Rocher, C., Hardy, S., Hubert, J.-F. and Le Rumeur, E. (2009) Mapping of the lipid-binding and stability properties of the central rod domain of human dystrophin. *J. Mol. Biol.* 389, 546–558.
- [41] Vie, V. et al. (2010) Specific anchoring modes of two distinct dystrophin rod sub-domains interacting in phospholipid Langmuir films studied by atomic force microscopy and PM-IRRAS. *Biochim. Biophys. Acta* 1798, 1503–1511.
- [42] Czogalla, A., Grzymajlo, K., Jezierski, A. and Sikorski, A.F. (2008) Phospholipid-induced structural changes to an erythroid b-spectrin ankyrin-dependent lipid-binding site. *Biochim. Biophys. Acta* 1778, 2612–2620.
- [43] Sarkis, J. et al. (2011) Spectrin-like repeats 11–15 of human dystrophin show adaptations to a lipidic environment. *J. Biol. Chem.* 286, 30481–30491.
- [44] Sarkis, J., Vié, V., Winder, S.J., Renault, A., Rumeur, E.L. and Hubert, J.-F. (submitted for publication). Resisting sarcolemmal rupture: Dystrophin repeats increase Membrane-Actin stiffness.
- [45] Stamler, J.S. and Meissner, G. (2001) Physiology of nitric oxide in skeletal muscle. *Physiol. Rev.* 81, 209–237.
- [46] Brenman, J.A., Chao, D.S., Xia, H., Aldape, K. and Bredt, D.S. (1995) Nitric oxide synthase complexed with dystrophin and absent from skeletal muscle sarcolemma in Duchenne muscular dystrophy. *Cell* 82, 743–752.
- [47] Torelli, S., Brown, S.C., Jimenez-Mallebrera, C., Feng, L., Muntoni, F. and Sewry, C.A. (2004) Absence of neuronal nitric oxide synthase (nNOS) as a pathological marker for the diagnosis of Becker muscular dystrophy with rod domain deletions. *Neuropathol. Appl. Neurobiol.* 30, 540–545.
- [48] Chao, D. et al. (1996) Selective loss of sarcolemmal nitric oxide synthase in Becker muscular dystrophy. *J. Exp. Med.* 184, 609–618.
- [49] Adams, M.E., Mueller, H.A. and Froehner, S.C. (2001) In vivo requirement of the  $\alpha$ -syntrophin PDZ domain for the sarcolemmal localisation of nNOS and aquaporin-4. *J. Cell Biol.* 155, 113–122.
- [50] Lai, Y. et al. (2009) Dystrophins carrying spectrin-like repeats 16 and 17 anchor nNOS to the sarcolemma and enhance exercise performance in a mouse model of muscular dystrophy. *J. Clin. Invest.* 119, 624–635.

- [51] Goyenvall, A., Seto, J.T., Davies, K.E. and Chamberlain, J. (2011) Therapeutic approaches to muscular dystrophy. *Hum. Mol. Genet.* 20, R69–R78.
- [52] Nicolas, A., Lucchetti-Miganeh, C., Yaou, R.B., Kaplan, J.-C., Chelly, J., Leturcq, F., Barloy-Hubler, F. and Rumeur, E.L. (submitted for publication). Assessment of the structural and functional impact of in-frame mutations of the DMD gene using the tools included in the eDystrophin online database.
- [53] Pawson, T. and Nash, P. (2003) Assembly of cell regulatory systems through protein interaction domains. *Science* 300, 445–452.
- [54] Sha, B., Phillips, S.E., Bankaitis, V.A. and Luo, M. (1998) Crystal structure of the *Saccharomyces cerevisiae* phosphatidylinositol-transfer protein. *Nature* 391, 506–510.
- [55] Ponting, C.P., Blake, D.J., Davies, K.E., Kendrick-Jones, J. and Winder, S.J. (1996) ZZ and TAZ: new putative zinc fingers in dystrophin and other proteins. *TIBS* 21, 11–13.
- [56] Sodek, J., Hodges, R.S., Smillie, L.B. and Jurasek, L. (1972) Amino-acid sequence of rabbit skeletal tropomyosin and its coiled-coil structure. *Proc. Natl. Acad. Sci. U S A* 69, 3800–3804.
- [57] Castresana, J. and Saraste, M. (1995) Does Vav bind to F-actin through a CH domain? *FEBS Lett.* 374, 149–151.
- [58] Ron, D. et al. (1991) A region of proto-dbl essential for its transforming activity shows sequence similarity to a yeast cell cycle gene, CDC24, and the human breakpoint cluster gene, bcr. *New Biol.* 3, 372–379.
- [59] Tufty, R.M. and Kretsinger, R.H. (1975) Troponin and parvalbumin calcium binding regions predicted in myosin light chain and T4 lysozyme. *Science* 187, 167–169.
- [60] Mayer, B.J., Ren, R., Clark, K.L. and Baltimore, D. (1993) A putative modular domain present in diverse signaling proteins. *Cell* 73, 629–630.
- [61] Haslam, R.J., Koide, H.B. and Hemmings, B.A. (1993) Pleckstrin domain homology. *Nature* 363, 309–310.
- [62] Wiche, G., Becker, B., Lubert, K., Weitzer, G., Castanon, M.J., Hauptmann, R., Stratowa, C. and Stewart, M. (1991) Cloning and sequencing of rat plectin indicates a 466-kD polypeptide chain with a three-domain structure based on a central alpha-helical coiled coil. *J. Cell Biol.* 114, 83–99.
- [63] Stahl, M.L., Ferenz, C.R., Kelleher, K.L., Kriz, R.W. and Knopf, J.L. (1988) Sequence similarity of phospholipase C with the non-catalytic region of src. *Nature* 332, 269–272.
- [64] Mayer, B.J., Hamaguchi, M. and Hanafusa, H. (1988) A novel viral oncogene with structural similarity to phospholipase C. *Nature* 332, 272–275.
- [65] Bork, P. and Sudol, M. (1994) The WW domain: a signalling site in dystrophin? *Trends Biochem. Sci.* 19, 531–533.
- [66] Kusunoki, H., Minasov, G., MacDonald, R.I. and Mondragon, A. (2004) Independent movement, dimerization and stability of tandem repeats of chicken brain  $\alpha$ -spectrin. *J. Mol. Biol.* 344, 495–511.
- [67] Koenig, M. and Kunkel, L.M. (1990) Detailed analysis of the repeat domain of dystrophin reveals four potential hinge segments that may confer flexibility. *J. Biol. Chem.* 265, 4560–4566.
- [68] Menhart, N. (2006) Hybrid spectrin type repeats produced by exon-skipping in dystrophin. *Biochim. Biophys. Acta* 1764, 993–999.
- [69] Way, M., Pope, B., Cross, R.A., Kendrick-Jones, J. and Weeds, A.G. (1992) Expression of the N-terminal domain of dystrophin in *E. coli* and demonstration of binding to F-actin. *FEBS Lett.* 301, 243–245.
- [70] Rentschler, S., Linn, H., Deininger, K., Bedford, M.T., Espanel, X. and Sudol, M. (1999) The WW domain of dystrophin requires EF-hands region to interact with beta-dystroglycan. *Biol. Chem.* 380, 431–442.